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Reef Fish Species Diversity Using Environmental DNA Metabarcoding in Mansinam and Lemon Island Waters, Manokwari Regency

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ABSTRACT Biodiversity has an essential role in the stability of an ecosystem. A high level of biodiversity indicates a more stable and stable ecosystem. The decline in the quality of ecosystems such as coral reefs, seagrass beds, and mangrove forests due to anthropogenic factors and global warming threaten biodiversity. This study aims to determine the diversity of reef fish species using an environmental DNA (eDNA) approach in the waters of the Mansinam and Lemon, Manokwari Regency islands. The analysis results detected 158 individual fish comprising 26 species from 10 families (sequence identity 97-100%). The highest species abundance was found in the Pomacentridae Family. The Pomacentridae family is an ornamental fish species in coral reef ecosystems. In addition, several species from the families Serranidae, Caesionidae, Mullidae, Holocentridae, Balistidae, Scaridae, and *Labridae* were also detected; fish species from these families are catch targets for fishers with economic value. Assessment of fish species diversity using the Shannon-Wiener method. The diversity of fish species in the waters of the islands of Mansinam and Lemon is in the high category, namely 3.17. High diversity indicates a stable and stable ecosystem condition.

Keywords: Biodiversity; eDNA; metabarcoding; Mansinam and Lemon Island

INTRODUCTION

The diversity of fish species is essential to understand an aquatic ecosystem's productivity and health condition. Biodiversity is essential for evaluating existing environmental conditions (Ahn *et al.*, 2020). Biodiversity conditions are critical information for understanding ecological processes, including biomonitoring purposes (Leray *et al.*, 2013). The waters of Mansinam and Lemon islands have the potential for aquatic ecology that has been used for a long time by the local community.

The local community traditionally uses the coastal waters of Mansinam and Lemon islands as fishing grounds. In the coastal waters of the islands of Mansinam and Lemon, coral reefs and seagrass ecosystems can be found (Lefaan *et al.*, 2013; Dasmasela *et al.*, 2019). Lemon Island is an important habitat for Walking Sharks (*Hemiscyllium galei*). This is based on samples of Walking Sharks found at the Aquatic Resources Laboratory, University of Papua, and the data has not been published. Its location, which is not far from the city, puts the ecosystems at both locations under direct pressure from various urban activities, such as the entry of plastic waste, organic waste, and others. Dasmasela *et al*. (2019) explained that the average value of coral cover in the waters of Mansinam Island showed severe coral damage. There is no information on the level of diversity of reef fish species that has been scientifically reported at both locations using the environmental DNA (eDNA) approach.

Environmental DNA, defined as genetic material released from organisms into the environment, has become an appropriate tool for studying molecular biology and ecology over the last decade (Ahn *et al.*, 2020). Environmental DNA has been used for biomonitoring studies of coral reef fish communities (Gelis *et al.*, 2021; Zuhdi *et al.*, 2021), species diversity and composition of chordates, molluscs, and echinoderms (Madduppa *et al.*, 2021), and fish species diversity (Ahn *et al.*, 2020; Andriyono *et al.*, 2019; Andriyono *et al.*, 2021). Environmental DNA is a good alternative for analyzing fish species diversity (Thomsen *et al.*, 2012).

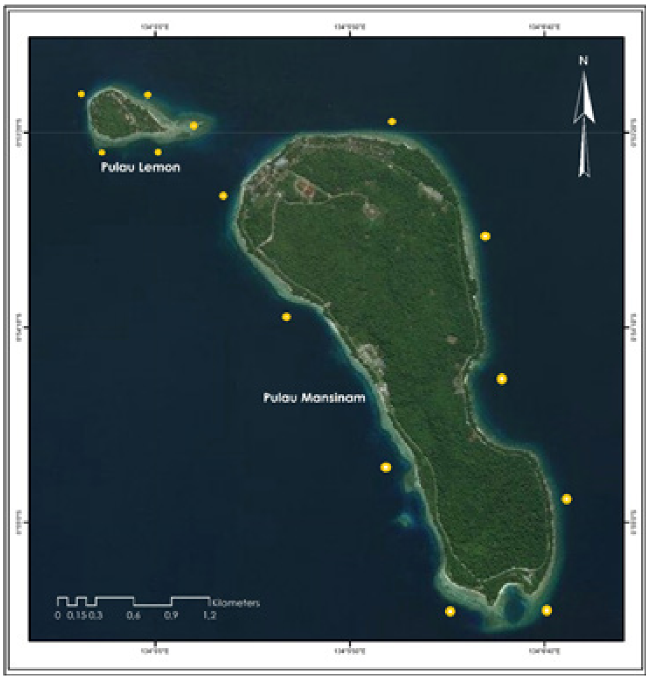
Fish release much genetic material from cells or faeces that can survive in the waters and then settle in sediments (Takahara *et al.*, 2013). Genetic material derived from organisms in water samples can be directly analyzed by Environmental DNA metabarcoding. This method has little impact on the ecosystem during sample collection (Yamamoto *et al.*, 2017; Bylemans *et al.*, 2019; McDevitt *et al.*, 2019) and must isolate the target organism (Laramie *et al.*, 2015).

This study aims to determine the diversity of fish species in the waters of the islands of Mansinam and Lemon. The approach used for species identification is using Environmental DNA metabarcoding.

MATERIALS AND METHODS

Research location and time

Sampling was carried out in the marine waters of Mansinam and Lemon Islands, Manokwari Regency, West Papua Province, Indonesia. Water sampling was carried out from March to April 2021 (Figure 1). Analysis of eDNA (extraction, amplification, electrophoresis, DNA sequencing) by laboratory assistant at Oceanogen Research Center, Bogor, Indonesia.



Note: (° Seawater sampling location)

Figure 1. Seawater sampling location map.

Water sampling technique

Seawater samples are collected to obtain fish genetic material. Seawater samples were taken using a Van Dorn Bottle Sampler with a capacity of 2 litres. The water sample as put into a 1.5-litre water bottle and stored in a cool box that had been given ice cubes.

The seawater samples were brought to the laboratory for filtration using a vacuum pump installed with Pall Corporation filter paper. Filters of type GN-6 Metricel MCE Membrane Disc Filters measuring 0.45 µm (Madduppa & Sani, 2020). After the filtering process is complete, the ilter paper is put into a 2 mL cryotube filled with a 1 mL DNA shield (Madduppa & Sani, 2020).

DNA extraction

DNA extraction breaks down the cell wall and separates the target DNA from unwanted cell particles, resulting in a pure DNA extract (Madduppa *et al.*, 2016). The process of the DNA extraction using the gSYNC™ Geneaid DNA Extraction Kit (Taipei, Taiwan) follows the manufacturer's protocol.

DNA amplification and electrophoresis

The first PCR amplifies the target region using MiFish primers (Forward and Reverse) (Miya *et al.*, 2015). The primers target a hypervariable region of the 12S rRNA gene (163–185 bp) (Miya *et al.*, 2015). MiFish primers demonstrated reliability for analyzing fish biodiversity from eDNA samples in marine (Yamamoto *et al.*, 2017; Ushio *et al.*, 2018) and freshwater (Sato *et al.*, 2018).

PCR was performed in 35 cycles with a 12 µl reaction volume containing 6.0 µl 2 × KAPA HiFi HotStart ReadyMix (including DNA polymerase, reaction buffer, dNTPs, and MgCl2 (at a final concentration of 2.5 mM)) (KAPA Biosystems, Wilmington, MA, USA), 0.7 µl each primer (5µM), 2.6 µl sterile distilled H_2O and 2.0 µl template (Miya *et al.*, 2015). Negative controls (i.e., blank templates) were used when running the Universal peqStAR 96 PCR machine (Peqlab Ltd, USA) to check for contamination.

The quality of the PCR products was visualized using electrophoresis on 2% agarose gel (100 mL TAE buffer and 2 g agarose). The electrophoresis machine was run at 50 Volts for 60 minutes, and the results were visualized using ultraviolet fluorescence via the Alphaimager Mini Gel Documentation System (Protein Simple Ltd, California, USA).

The first PCR product was diluted ten times using Milli-Q water as a template for the second PCR. The second PCR was performed with 12 cycles of 12 µl reaction volume containing 6.0 µl 2×KAPA HiFi HotStart ReadyMix, 0.7 µl each primer (5µM), 3.6 µl sterile distilled H2O, and 1.0 µl template (Miya *et al.*, 2015).

The thermal cycle profile after an initial 3 min denaturation at 95°C is as follows: denaturing at 98°C for 20 seconds; annealing and extension were combined at 72°C (shuttle PCR) for 15 seconds with a final extension at the same temperature for 5 min (Miya *et al.*, 2015).

DNA sequencing

Library concentrations were estimated using the Qubit dsDNA HS assay kit and Qubit fluorometer (Life Technologies) (Miya *et al.*, 2015). The double-stranded DNA concentration from the pooled library was adjusted to 4 nM (assuming one bp equals 660 g mol−1) using Milli-Q water, and 5 l from the 4 nM library was denatured with 5 l NaOH 0.1 N (Miya *et al.*, 2015). Including the HT1 buffer (provided by the Illumina MiSeq Reagent v. 2 kits for 2 × 150 bp PE), the denatured library (10 l; 2 nM) was diluted to a final concentration of 12 m for sequencing on the MiSeq platform. 30 l of control DNA spike PhiX (12 m) was added to improve the data quality of low diversity samples, such as the single PCR amplicon used in this study (Miya *et al.*, 2015).

Bioinformatics

Bioinformatics analysis using Pipeline MiFish (Sato *et al.*, 2018). The MiFish pipeline was used to analyze FASTQ files produced by iSeq 100 (http://mitofish.aori.u-tokyo.ac.jp/mifish/)

Diversity assessment

Assessment of fish species diversity using the Shannon-Wiener equation with the following formula:

$$H' = -\sum_{i=1}^{R} pi \ln pi = -\sum_{i=1}^{R} \ln pi^{pi}$$

Description:
Pi = ni/N
ni = number of fish for species- i
N = Total individual fish for all species.

RESULTS AND DISCUSSION

The total detected reef fish were 158 individuals consisting of 26 species from 10 families (Figure 2). The similarity between the sequences of the sequencing results with the database is 97-100%. The results of BLASTN sequences 97-100% showed a significant level of sequence similarity (Bhattacharjee *et al.*, 2012).

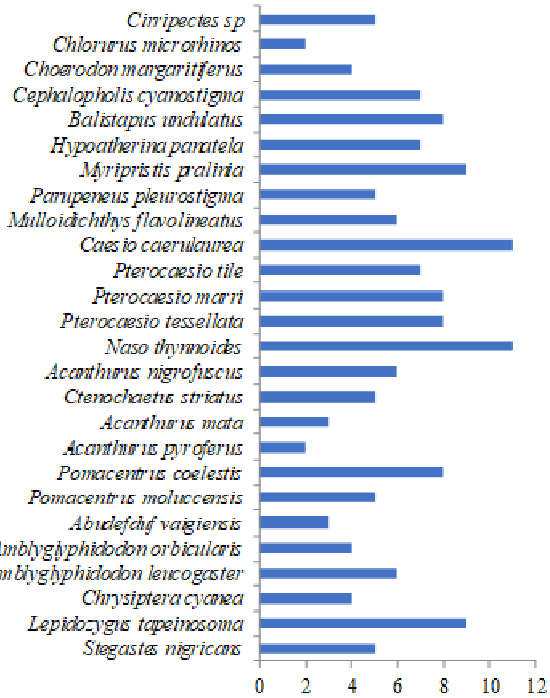


Figure 2. Fish species abundance.

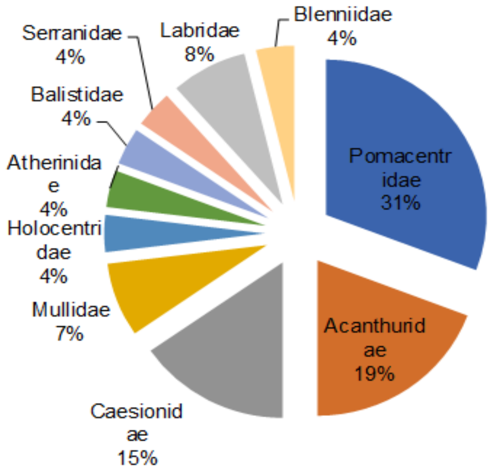
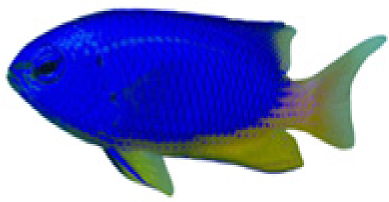


Figure 3. The composition of fish species in each family.

The highest species composition was found in the Pomacentridae family (Figure 3). The Pomacentridae family is an ornamental fish found living on coral reefs. Fish of the Pomacentridae family detected in the waters of the islands of Mansinam and Lemon were 44 individuals from 8 species. Eight species are Stipetes *nigricans*, *Lepidozygus tapeinosoma*, *Chrysiptera cyanea*, *Amblyglyphidodon leucogaster*, *Amblyglyphidodon orbicularis*, *Abudefduf vaigiensis*, *Pomacentrus moluccensis*, and *Pomacentrus coelestis* (Figure 4).

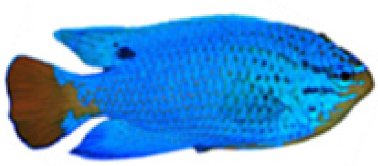
Stegastes nigricans (https://fishesofaustralia.net.au/).



Pomacentrus coelestis (Bray, 2022).

Lepidozygus tapeinosoma (https://fishesofaustralia.net.au/).

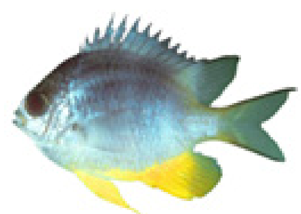
Pomacentrus moluccensis (Bray, 2022).



Chrysiptera cyanea (https://fishesofaustralia.net.au/).

Abudefduf vaigiensis (White *et al*., 2013).

Amblyglyphidodon leucogaster (Allen, 2012).



Amblyglyphidodon orbicularis (Allen, 2002).

Figure 4. Pomacentridae family.

The Pomacentridae family is one of the major fish groups in coral reef ecosystems (English, 1997; Rondonuwu *et al*., 2013). Major fish have relatively small body sizes (Falah *et al*., 2020). The Pomacentridae family plays a vital role in the food chain, especially as a food supply for carnivorous fish (Wibowo, 2013).

Fish species of the Pomacentridae family are found mainly in tropical seas and some in the subtropics (Suwartimah *et al*., 2016). Most species of the Pomacentridae family are found on or near coral reefs in the Indo-West Pacific (Nelson, 1994). The territorial waters from the Philippines to Australia have the most prominent species concentrations (Nelson, 2006). Most species of the Pomacentridae family live in shallow water, from 2 to 15 meters deep. However, some species are found at 100 meters depth (Pyle *et al*., 2008).

Several similar research results show that the Pomacentridae family has a high abundance in the Raja Ampat Islands (McKenna, 2002), Pamuteran and Sumber Kima waters, Buleleng, Bali (Suwartimah *et al*., 2016), Halang Island Melingkau South Kalimantan (Tony *et al*., 2020), the Redang islands of Malaysia (Du *et al*., 2019) and Unggeh Island, Central Tapanuli Regency, North Sumatra, Indonesia (Harahap & Susetya, 2020). Pomacentridae is one of the most abundant reef fish; about 340 species can live in coral reef environments (Frederich *et al*., 2009).

The abundance of species from the Pomacentridae family in the waters can be associated with good coral reef ecosystem conditions and feeding habits (Falah *et al*., 2020). The high abundance of fish from the Pomacentridae family is caused by the condition of the coral reefs, which are still classified as very good for the Pomacentridae family (Allen 2012). Fish species in the Pomacentridae family utilize plankton and algae in coral reef ecosystems as food (Ratnawati *et al*., 2011). In addition, the Pomacentridae family has the highest number of species and is widely distributed in coral reef ecosystems worldwide (Yanuar, 2015). The abundance of the Pomacentridae family is supported by the condition of the coral reef ecosystem on Mansinam and Lemon Islands, which is classified as good (Dasmasela *et al*., 2019) and the physical-chemical conditions of the waters that meet the quality standards (Wyzer *et al*., 2018). In addition, the abundance of the Pomacentridae family is caused by the behaviour of fish that are resident species in their habitat and are territorial fish that always flock in large numbers. The Pomacentridae family is not the target fish for fishing on Mansinam and Lemon Islands, so its abundance in nature is maintained compared to other families in this study which are low in abundance.

Fish species from the Acanthuridae family have the highest abundance after the Pomacentridae family. The number of individuals of the Acanthuridae family is 27 Ind which comes from 5 species. Five of these species are *Acanthurus pyroferus*, *Acanthurus mata*, *Acanthurus nigrofuscus*, *Ctenochaetus striatus*, and *Naso thynnoides* (Table 2). The Acanthuridae family lives in coral reef ecosystems (Bellwood *et al*., 2014). Most fish from the family Acanthuridae feed on zooplankton, detritus, and benthic algae (Nelson, 1994). Fish species of the Acanthuridae family affect the structure of coral reef substrates through competition, abundance, and algal dynamics. McClanahan (2001) explained that reef fish could control the number of algae on the substrate, thus allowing corals to grow faster to develop their colonies.

Acanthurus pyroferus (White *et al*., 2013).

Ctenochaetus striatus (White *et al*., 2013).

Acanthurus mata (White *et al*., 2013).

Naso thynnoides (White *et al*., 2013).

Acanthurus nigrofuscu (White *et al*., 2013).

Figure 5. Acanthuridae family.

The detected Serranidae family was a grouper of the *Cephalopholis cyanostigma* species (Figure 4). In Indonesia, Grouper has commercial value (Yulianto *et al*., 2015; Syafei, 2018; Maulida *et al*., 2020) and is the main target fish for fishers. The grouper group belongs to the family Serranidae, which naturally inhabits shallow-water habitats in coral reefs, estuaries, mangroves, and seagrasses in both tropical and subtropical areas (Kamal *et al*., 2019).

There are 76 species of grouper found in Indonesian waters (Choat, 2018). Most of the groupers found in Indonesia belong to the genus Epinephelus (Tapilatu *et* al., 2021). Three species are in the "vulnerable" category, five are "Data Deficient," and 68 species are in the "Least Concern" category (Choat, 2018). Cephalopholis cyanostigma is included in the "Least Concern" category with a stable natural population trend (Choat, 2018).

Figure 6. *Cephalopholis cyanostigma* (White *et al.*, 2013).

The Caesionidae family are coral-associated fish, non-migratory, distributed throughout the Indo-West Pacific region, from Sri Lanka to Vanuatu and from southern Japan to northern Australia (Froese, 2021). The family Caesionidae is predominantly coral reef fish species and occurs near the surface to a depth of 60 m (200 ft) (Carpenter, 2001). Fish species from the Caesionidae family are zooplankton eaters in the water column, often found in coral reef ecosystems (Rosdianto *et al.*, 2021). Fish species of the Caesionidae family are the target fish for fishers' catch and have economic value, so the abundance is not so high. Four species were found in the waters of Mansinam and Lemon islands: Pterocaesio tessellate, Pterocaesio tile, *Caesio caerulaurea*, and *Pterocaesio marri* (Table 3).

Pterocaesio tessellate (White *et al.*, 2013).

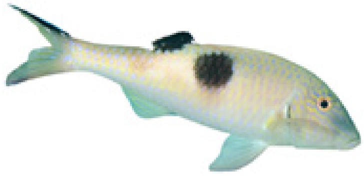
Pterocaesio tile (White *et al.*, 2013).

Caesio caerulaurea (White *et al.*, 2013).

Pterocaesio marri
(https://fishesofaustralia.net.au/).

Figure 7. Caesionidae family.

Families Mullidae, Holocentridae, Balistidae, Scaridae, and Labridae were detected in the waters of the Lemon and Mansinam islands. However, the number of species was not significant. The types of fish from this family are the target fish for fishers to catch for sale or consumption. Descriptions of species from the families Mullidae (Figure 6), Holocentridae (Figure 9), Balistidae (Figure 10), Scaridae (Figure 11), and Labridae (Figure 12).



Parupeneus pleurostigma (https://fishesofaustralia.net.au/).

Mulloidichthys flavolineatus (White *et al.*, 2013).

Figure 8. Mullidae family.

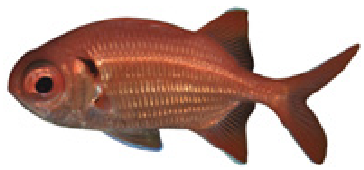


Figure 9. *Myripristis pralinia* (https://fishesofaustralia.net.au/).

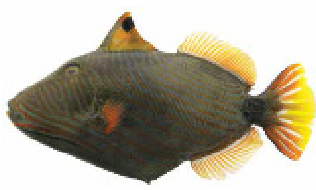


Figure 10. *Balistapus undulates* (White *et al.*, 2013)

Figure 11. *Chlorurus microrhinos* (White *et al.*, 2013).

Figure 12. *Choerodon margaritiferus* (White *et al.*, 2013).

The diversity index is a quantitative measure of how many different species are in a community (Tucker *et al.*, 2017). The Shannon index is widely used in diversity studies in the ecological literature (Spellerberg, 2003). Based on the analysis of the fish species diversity index in the waters of the islands of Mansinam and Lemon, it is 3.17. The diversity index value is in the high category, meaning that the diversity of fish species in the waters of Mansinam and Lemon islands is very high. Hukom (1998) grouped the diversity index values as low (H' < 2.0), medium (2.0 < H' < 3.0), and high (H' > 3.0). The diversity value is still higher compared to the assessment of reef fish species diversity in Gof Kecil and Yep Nabi Raja Ampat islands, namely 2.47 and 2.98 (Panggabean *et al.*, 2012).

The high index of fish species diversity in the waters of Mansinam and Lemon islands indicates high water productivity. Water conditions with good productivity support the high diversity of species resources. The research results by Ayhuan *et al*. (2017) found a high index of macroalgae diversity in the waters of the islands of Mansinam and Lemon. In addition, in the waters of the islands of Mansinam and Lemon, we can find several ecosystems, such as coral reefs and seagrass beds that function as ecosystems for vertebrate and invertebrate biota. Based on Dasmasela *et al*. (2019), the condition of coral reefs on Mansinam Island is considered good. However, it is under pressure from destructive human activities. Based on Nybakken (1992), the high species diversity in coral reefs is due to the variety of coral reef habitats, consisting of coral and sandy areas, various bays and crevices, algae, and shallows. The types of coral reefs that can be found on Mansinam Island and are habitats for the composition of fish species found in this study consist of Acropora Digitatae, Acropora Tabulate, Coral Encrusting, Coral Foliiose, Coral Heliopora, Coral Juvenile, Coral Millepora, Coral Musroom, Coral Submasive, Old Dead Coral, Soft Coral, Sponges, Marcoalgae and True Algae (Dasmasela *et al.*, 2019).

The condition of coral reef ecosystems affects reef fish diversity (Rosdianto *et al.*, 2021) because coral reefs function as shelters and foraging places for food (Pratchett, 2008). In addition, McClure *et al*. (2021) explained that the abundance of reef fish depends on the condition of the coral reefs and the complexity of the habitats that exist in the ecosystem. Coral reef ecosystems function as a place to live, find food, and breed for types of reef fish (Rosdianto *et al.*, 2021). So that the condition of damaged coral reefs causes fewer fish species to live because their habitat is no longer able to meet food stocks and breeding grounds (Hempson *et al.*, 2017).

CONCLUSIONS

The total fish detected were 158 individuals consisting of 26 species from 10 families. Based on the assessment results of the diversity of fish species in the waters of the islands of Mansinam and Lemon, they are in the high category. High diversity indicates ecological conditions that have high productivity.

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